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(54) Title: METHODS OF MODULATING BLOOD PRESSURE USING TGF-β AND ANTAGONISTS THEREOF

(57) Abstract

The use of TGF-β and TGF-β antagonists to modulate blood pressure is described. In a specific embodiment described by way of example herein, recombinant mature TGF-β1 isolated and purified from transfected Chinese Hamster Ovary cells induced rapid, significant and sustained decreases in arterial blood pressure of cynomolgus monkeys receiving daily injections of the rTGF-β1. The TGF-β used to lower blood pressure may be obtained from native sources or may be produced by recombinant DNA or chemical synthetic techniques.

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METHODS OF MODULATING BLOOD PRESSURE USING TGF-β AND ANTAGONISTS THEREOF

1. INTRODUCTION

The present invention is directed to the use of transforming growth factor-beta (TGF-β) and TGF-β antagonists to modulate blood pressure (BP). The method of the invention is demonstrated by way of example in which mature recombinant TGF-β1 (rTGF-β) is used to rapidly lower blood pressure in adult cynomolgus monkeys. However, the scope of the invention is not limited to the use of rTGF-β1 but rather encompasses the use of mature and precursor forms of all members of the TGF-β family effective at modulating blood pressure, including natural and recombinant mature TGF-β1, TGF-β2, TGF-β3, TGF-β4, etc., as well as TGF-β hybrids, analogs and latent TGF-β complexes. Similarly, the invention includes the use of any and all compositions effective at antagonizing TGF-β activity, including but not limited to anti-TGF-β antibodies and TGF-β receptors.

2. BACKGROUND OF THE INVENTION

2.1. TRANSFORMING GROWTH FACTOR-BETA

TGF-β is a member of a recently described family of polypeptides that regulate cellular differentiation and proliferation. Other members of this family include Mullerian inhibitory substance (Cate et al., 1986, Cell 45:685-698), the inhibins (Mason et al., 1985, Nature 318:659-663) and a protein predicted from a transcript of the decapentaplegic gene complex of Drosophila (Padgett et al., 1987, Nature 325: 81-84.

Four types of TGF-β have been identified and designated TGF-β1, TGF-β2, TGF-β1.2, and TGF-β3. The first described type, TGF-β1, consists of two identical disulfide linked subunits having molecular weights of 13,000 (Assoian et al., 1983, J. Biol. Chem. 258:7155-7160; Frolik et al.,

1983, Proc. Natl. Acad. Sci. USA 80:3676-3680; Frolik et al., 1984, J. Biol. Chem. 260:10995-11000). It has been purified from several tissue sources including placenta (Frolik et al., 1983, Nature 325:81-84), blood platelets (Childs et al., 1982, Proc. Natl. Acad. Sci. USA 79:5312-5316; Assoian et al., 1983, J. Biol. Chem. 258:7155-7160) kidney (Roberts et al., 1983, Biochemistry 22:5692-5698), and demineralized bone (Seyedin et al., 1985, Proc. Natl. Acad. Sci. USA 82:119-123). cDNA clones coding for human (Derynck et al., 1985, Nature 316:701-705), mouse (Derynck et al., 1986, J. Biol. Chem. 261:4377-4379) and simian (Sharples et al., 1987, DNA 6:239-244) TGF-β1 have been isolated. DNA sequence analysis of these clones indicates that TGF-β1 is synthesized as a large precursor polypeptide, the carboxy terminus of which is cleaved to yield the mature TGF-β monomer. Strong sequence homology has been found throughout the TGF-β1 precursor protein from all of the above sources.

In the presence of 10% serum and epidermal growth factor, TGF-β1 promotes the anchorage independent growth of normal rat kidney fibroblasts (Roberts et al., 1981, Proc. Natl. Acad. Sci. USA 78:5339-5343; Roberts et al., 1982, Nature 295:417-419; Twardzik et al., 1985, J. Cell. Biochem. 28:289-297); in the presence of 10% serum alone, it is able to induce colony formation of AKR-2B fibroblasts (Tucker et al., 1983, Cancer Res. 43:1518-1586). TGF-β1 has also been shown to cause fetal rat muscle mesenchymal cells to differentiate and produce cartilage specific macromolecules (Seyedin et al., 1986, J. Biol. Chem. 261:5693-5695).

In contrast to its effect on cell proliferation, TGF-β1 purified from human platelets has been shown to inhibit the growth of certain cells in culture (Tucker et al., 1984, Science 226:705-707). TGF-β1 has also been shown to inhibit the growth of several human cancer cell lines

(Roberts et al., 1985, Proc. Natl. Acad. Sci. USA 82:119-123). This inhibitory/stimulatory effect of TGF-β1 may depend on several factors including cell type and the physiological state of the cells (for review see Sporn et al., 1986, Science 233:532-534).

TGF-β2, like TGF-β1, is a polypeptide of molecular weight 26,000 composed of two identical 13,000-dalton subunits which are disulfide like (Chiefetz et al., 1987, Cell 48:409-415; Ikeda et al., 1987, Biochemistry 26:2406-2410) and has been isolated from bovine demineralized bone (Seydin et al., 1987, J. Biol. Chem. 262:1946-1949), porcine platelets Chiefetz et al., 1987, 48:409-415), a human prostatic adenocarcinoma cell line, PC-3 (Ikeda et al., 1987, biochemistry 26:2406-2410), and a human gliablastoma cell line (Wrann et al., 1987, EMBO 6:1633-1636). cDNA clones coding for human and simian TGF-β2 have been isolated (Madisen et al., 1988, DNA 7:1-8; Webb et al., 1988, DNA 7:493-497). The mature TGF-β2 monomer is cleaved from one of two larger precursor polypeptides, the mRNAs of which may arise via differential splicing (Webb et al., 1988, DNA 7:493-497).

TGF-β1 and TGF-β2 share 71% amino acid sequence identity in their mature regions, and 41% identity in their precursor structures. TGF-β3, the amino acid sequence of which has very recently been deduced from cDNA clones, appears to contains C-terminal 112 amino acid sequence with about 80% homology to the mature monomers of TGF-β1 and TGF-β2 (Dijke et al., 1988, Proc. Natl. Acad. Sci. USA 85:4715-4719). TGF-β1.2 is a heterodimeric form comprising a β1 and β2 subunit linked by disulfide bonds (Chiefetz et al., 1987, Cell 48:409-415).

3. SUMMARY OF THE INVENTION

The present invention is directed to methods of modulating blood pressure using TGF-β polypeptides, TGF-β antagonists, and/or combinations thereof. The invention may be subdivided into two categories solely for the purpose of description.

First, the invention relates to the use of TGF-βs as antihypertensive agents capable of rapidly and significantly lowering blood pressure. This aspect of the invention encompasses the use of any and all TGF-β polypeptides having a hypotensive activity, including mature and precursor forms of TGF-β1, TGF-β2, TGF-β3, hybrid TGF-βs, latent TGF-β complexes, TGF-β analogs, etc. In a specific embodiment of the invention, described more fully by way of example herein (Section 6., infra), simian recombinant TGF-β1 is administered parenterally to induce rapid significant, and sustained decreases of arterial blood pressure in cynomolgus monkeys. In a related embodiment, TGF-βs may be used to rapidly lower blood pressure to normal levels in patients facing acute hypertension and emergency conditions associated with extreme hypertension.

Second, the invention relates to the use of TGF-β antagonists to elevate blood pressure through the inhibition of hypotension induced by TGF-β and/or related factors. Any composition which antagonizes TGF-β activity may be useful in this regard, including for example, anti-TGF-β antibodies and TGF-β receptors. Additionally, methods which lower and/or maintain the level of circulating TGF-β in an individual may result in a similar pressor effect. For example, anti-TGF-β antisense RNA molecules may inhibit synthesis and release of bioactive TGF-βs, thereby preventing excessive hypotensive signal generation and resulting hypotension.

4. DESCRIPTION OF THE FIGURES

FIG. 1. Nucleotide sequence of simian TGF-β1 cDNA and deduced amino acid sequence. The 1600 bp insert of pTGF-β1-2 was subcloned into the M13mp18 and M13mp19 cloning vectors (Yanisch-Perron et al., 1985, Gene 33:103-119) and both strands were sequenced using the dideoxy chain-termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. USA 74:5463-5467). The deduced amino acid sequence of simian TGF-β1 is presented directly above the cDNA sequence. The human TGF-β1 nucleotide sequence is aligned with and presented directly below the simian cDNA sequence; dots indicate homologous nucleotide residues within the sequences. Amino acid differences between the human and simian proteins are indicated in the top line. The mature TGF-β1 sequence is boxed and the signal peptide is overlined.

FIG. 2. Nucleotide sequence of human TGF-β2-442 cDNA and deduced amino acid sequence. The 2597 BP insert of PC-21 was subcloned into pEMBL (Dante et al., 1983, Nucleic Acids Res. 11:1645-1654) and sequenced on both strands using the dideoxy chain-termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. USA 74:5463-5467). The coding sequence is shown and the deduced amino acid sequence is presented directly above. The mature TGF-β2 sequence is boxed and the signal peptide is overlined. Potential glycosylation sites are indicated by asterisks. The arrow indicates the putative signal sequence cleavage site. The nucleotide sequence of simian TGF-β2-414 cDNA is identical to the human TGF-β2-442 cDNA sequence except that (a) nucleotides 346 through 432 (bracketed) are deleted and replaced by the sequence AAT, and (b) several silent nucleotide changes occur elsewhere in the structure (indicated by single letters directly below the changed nucleotide). The deduced amino acid sequence for simian

TGF-β2-414 precursor is identical to the human TGF-β2-442 precursor amino acid sequence except that Asparagine replaces amino acid residues 116 through 144 in the human TGF-β2-442 structure. The nucleotide sequence of a human TGF-β2-414 cDNA has been sequenced through the region indicated by broken underlining and was found to be perfectly homologous to the human TGF-β2-442 cDNA sequence except that nucleotides 346 through 432 are deleted and replaced by the sequence AAT.

FIG. 3. Nucleotide sequence of hybrid TGF-β1/β2 precursor DNA and deduced amino acid sequence. The coding sequence is shown and the deduced amino acid sequence is presented directly above. The mature TGF-β2 sequence is boxed and the precursor signal peptide is overlined. Glycosylation sites are indicated by asterisks. The arrow indicates the putative signal sequence cleavage site. The TGF-β2 mature coding sequence depicted is of human origin. The simian TGF-β2 mature coding sequence is nearly identical to the human sequence: only 3 silent base changes occur and are indicated by single letters directly below the changed nucleotide.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to methods of modulating blood pressure in an animal using TGF-β polypeptides, antagonists and/or combinations thereof. The invention is based upon the discovery that parenterally administered mature rTGF-β1 rapidly and significantly lowers blood pressure in cynomologus monkeys. Thus, one aspect of the invention relates to the use of TGF-βs as antihypertensive/hypotensive agents. Precisely the opposite effect, i.e., raising and/or maintaining blood pressure, may be achieved by TGF-β antagonists capable of inhibiting the antihypertensive/hypotensive effects of TGF-β. In this

regard, the invention encompasses the use of anti-TGF-β antibodies, TGF-β receptors and other compositions capable of inhibiting TGF-β-induced hypotension.

5.1. USE OF TGF-βs AS ANTIHYPERTENSIVE AGENTS

One aspect of the invention relates to the use of TGF-βs as antihypertensive/hypotensive agents. Applicants' initial data indicates that rTGF-β1 can rapidly and significantly lower blood pressure in simian test subjects at a dosage which appears to be at or close to the physiologically tolerable limit. In this regard, parenteral administration of a TGF-β at such a dose may be acceptable in hypertensive emergencies requiring agressive treatment. Lower does of a TGF-β may also be effective at reducing blood pressure, and such doses may be appropriate for patients with moderate to severe hypertension. In these patients, less agressive therapy may be desirable where adverse side effects can not be tolerated.

Human patients with diastolic blood pressure greater than 130 mm Hg and complications such as hypertensive encephalopathy, progressive renal failure, acute pulmonary edema, cerebral accident, papilledema, or multiple fresh retinal hemorhages are generally treated agressively with a parenteral antihypertensive agent such as, for example, nitroprusside and diazoxide. Treatment of hypertension characterized by such acute complications generally aims to lower BP to about 100 mm Hg within 30 to 60 minutes, since rapid decrease is a key determinant of survival in patients facing these emergencies.

The TGF-β anithypertensive may be administered alone or in combination with other antihypertensive agents in suitable pharmacological carriers via any appropriate route. In hypertensive emergencies, parenteral administration will provide the fastest decrease in BP and

is therefore the recommended route of administration in such situations. Additionally, the TGF-β may be linked to a carrier or targeting molecule and/or incorporated into liposomes, microcapsules, and controlled release preparations prior to administration in vivo.

5.1.1. SOURCES OF TGF-β

In accordance with the invention, mature and/or precursor forms of TGF-β1, TGF-β2, TGF-β3, TGF-β1/β2, etc., may be used to lower blood pressure. The TGF-β used may be obtained from a variety of sources, including but not limited to isolating natural TGF-βs from appropriate sources, producing TGF-β by recombinant DNA techniques, or by chemical synthetic methods, etc.

5.1.1.1. TGF-β1

Natural TGF-β1 can be isolated from a variety of sources. This potent modulator of cell behavior is synthesized by a variety of normal and transformed cells in culture (Roberts et al., 1981, Proc. Natl. Acad. Sci. USA 78:5339-5343) and has been purified from various sources including placenta (Frolik et al., 1983, Proc. Natl. Acad. Sci. USA 80:3676-3680), kidney (Roberts et al., 1983, Biochemistry 22:5692-5698), urine (Twardzik et al., 1985, J. Cell. Biochem. 28:289-297) and blood platelets (Childs et al., 1982, Proc. Natl. Acad. Sci. USA 79:5312-5316). Additionally, the human (Derynck et al., 1985, Nature 316:701-705), mouse (Derynck et al., 1986, J. Biol. Chem. 261:4377-4379), and simian (Sharples et al., 1987, DNA 6:239-244) TGF-β1 have been described.

Large quantities of TGF-β1 may be obtained by recombinant DNA techniques using eucaryotic host cells transfected with recombinant DNA vectors containing the

TGF-β1 coding sequence controlled by expression regulatory elements. Examples of such methods are described in copending application Serial No. 07/353,728 filed August 17, 1989, which application is incorporated by reference herein it its entirety. Briefly, a cDNA clone coding for simian TGF-β1 precursor was obtained from a cDNA library made from an African Green Monkey cell line, BSC-40. The deduced amino acid sequence of the mature simian TGF-β1 shown in FIG. 1 has 100% homology with that of the mature human TGF-β1. Expression vectors were constructed which contain the entire coding sequence for the simian TGF-β1 precursor placed under the control of SV40 expression elements. They were used to transfect Chinese Hamster Ovary cells (CHO cells). The resulting CHO transfectants produce and secrete primarily a high molecular weight complex from which mature bioactive TGF-β may be liberated by a routine acidification procedure.

5.1.1.2. TGF-β2

Natural TGF-β2 used in accordance with the invention can be obtained from a variety of sources. A protein isolated from bovine demineralized bone has been identified as being related to TGF-β (Seyedin et al., 1987, J. Biol. Chem. 262:1946-1949). The protein has also been isolated from porcine platelets (Cheifetz et al., 1987, Cell 48:409-415), a human prostatic adenocarcinoma cell line PC-3 (Ikeda et al., 1987, Biochemistry 26:2406-2410), and a human glioblastoma cell line (Wrann et al., 1987, EMBO 6:1633-1636). Partial amino acid sequence of this protein indicated that it was homologous to TGF-β and has been termed TGF-β2.

Large quantities of TGF-β2 may be obtained by recombinant DNA techniques using eukaryotic host cells transfected with recombinant DNA vectors containing a TGF-β2 coding sequence controlled by expression regulatory

elements. Examples of such methods are described in copending application Serial No. 07/446,020 filed December 5, 1989, which application is incorporated by reference herein in its entirety. Briefly, cDNA clones coding for human TGF-β2 precursor were obtained from a cDNA library made from a tamoxifen treated human prostatic adenocarcinoma cell line, PC-3. The cDNA sequence of one such clone is shown in FIG. 2 and predicts that TGF-β2 is synthesized as a 442 amino acid polypeptide precursor from which the mature 112 amino acid TGF-β2 subunit is derived by proteolytic cleavage. This TGF-β2 precursor, termed TGF-β2-442, shares a 41% homology with the precursor of TGF-β1. In another embodiment, cDNA clones coding for simian TGF-β2 precursor were obtained from a cDNA library made from an African green monkey kidney cell line, BCS-40. The cDNA sequence of one such clone predicts that TGF-β2 is also synthesized as a 414 amino acid polypeptide precursor from which the mature 112 amino acid TGF-β2 subunit is derived by proteolytic cleavage. This TGF-β2 precursor, termed TGF-β2-414, has an amino acid sequence of 414 amino acid residues and is identical to the amino acid sequence of TGF-β2-442, except that it contains a single Asparagine residue instead of the 29 amino acid sequence from residue numbers 116 to 135 of the human TGF-β2-442 sequence.

Clones from the BSC-40 cDNA library which encode a simian TGF-β2-442 precursor as well as clones from the human PC-3 cDNA library which encode a human TGF-β2-414 precursor have also been identified. The human and simian TGF-β2-442 precursors appear to be perfectly homologous at the amino acid level, as do the human and simian TGF-β2-414 precursors.

5.1.1.3. HYBRID MATURE AND PRECURSOR TGF-βs

Hybrid mature TGF-β molecules may be prepared using recombinant DNA techniques or synthetic methods. Examples of such methods are also described in copending applications Serial No. 284,972, filed December 15, 1988, which application is incorporated by reference herein in its entirety.

Hybrid precursor TGF-β molecules may be prepared using recombinant DNA techniques or synthetic methods, as described in co-pending application Serial No. 07/353,728 filed August 17, 1987. Briefly, expression vectors containing the TGF-β2 mature coding sequence joined in-phase (i.e., in the same translational reading frame) to the TGF-β1 signal and precursor sequences (see FIG. 3) were constructed and used to transfect Chinese Hamster Ovary cells (CHO cells). The resulting CHO transfectants produce and secrete mature, biologically active TGF-β2.

5.1.1.4. MODIFIED TGF-β

Variations in the amino acid sequences shown herein for the different TGF-β molecules, as well as variations in the steric configuration, the type of covalent bonds which link the amino acid residues, and/or addition of groups to the amino- or carboxy-terminal residues are within the scope of the invention. For example, the TGF-β molecules used in accordance with the invention may include altered sequences such as conservative alterations which result in a silent change thus producing a functionally equivalent molecule. Thus, the amino acid sequences shown in FIGS. 1-3 may be altered by various changes such as insertions, deletions and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use. As used herein, conservative substitutions would involve the substitution of

one or more amino acids within the sequences shown with another amino acid having similar polarity and hydrophobicity/hydro-philicity characteristics resulting in a silent alteration and a functionally equivalent molecule. Such conservative substitutions include but are not limited to substitutions within the following groups of amino acids: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; phenylalanine, tyrosine; and methionine, norleucine.

5.1.1.5. LATENT TGF-β COMPLEX

TGF-β1 may be isolated from tissues or tissue culture cells in an inactive, biologically latent form which may be activated by chaotropic agents, proteases, or in vivo. Similarly, CHO cell transfected with the simian TGF-β1 precursor coding sequence secrete a high molecular weight latent complex involving both the mature and "pro" regions of the TGF-β precursor. The association of the "pro" region of the TGF-β precursor has also been observed in latent TGF-β1 complex isolated from platlets. Although the mechanism of activation in vivo is unknown, it is possible that the latent complex provides an important level of regulation on TGF-β1 bioactivity. In accordance with the method of the invention, latent TGF-β complex may be useful as a means of controlling the hypotensive effect induced by the bioactive form of TGF-β by releasing it at the situs of natural in vivo activation mechanisms. The identification, isolation and characterization of latent TGF-β1 complex from recombinant CHO cells is described more fully in copending application Serial No. 07/353,728 filed August 17, 1989, which application is incorporated herein by reference in its entirety.

5.2. USE OF TGF-β ANTAGONISTS AS PRESSOR AGENTS

Applicants' discovery that rTGF-β1 is capable of rapidly and significantly lowering blood pressure suggests that the TGF-βs may be involved in the regulation of BP and/or in the genesis of hypotension. In this regard, through their ability to impede the hypotensive effect of TGF-β, antagonists of TGF-βs may be useful as pressor/hypotensor agents capable of elevating BP. Any compostion which effectively antagonizes the hypotensive effect of a TGF-β may be used for this purpose, including but not limited to anti-TGF-β antibodies and TGF-β receptors.

For example, TGF-β antagonists may be useful in treating medical conditions characterized by a loss of BP where the elevation of BP to normal levels is desirable. Such conditions include, for example, shock associated with blood volume loss, cardiac emergencies, and hypotension in acute renal failure. The TGF-β antagonists may be administered alone or in combination and/or together with other pressors/hypotensors such as dopamine, epinephrine, aminophylline, etc. Compounds containing effective doses of TGF-β antagonist formulated in a suitable pharmacological carrier may be administered to patients experiencing hypotension or conditions associated with hypotension via any appropriate route including but not limited to injection, infusion and selective catheterization in order to elevate BP. In addition, the TGF-β antagonist may be linked to a carrier or targeting molecule and/or incorporated into liposomes, microcapsules, and controlled release preparations prior to administration in vivo.

5.2.1. ANTI-TGF-β ANTIBODIES

Antibodies capable of inhibiting the hypotensive effect of TGF-β may be useful as pressor agents. Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of TGF-βs. For the production of antibodies, various host animals can be immunized by injection with a TGF-β, or a synthetic TGF-β peptide, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peotides, oil emulsions, keyhold lympet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

A monoclonal antibody to an epitope of a TGF-β can be prepared by using any technique which provides for the production of antibody molecules by continous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256, 495-497), and the more recent human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Antibody fragments which contain the idiotype of the molecule may be generated by known techniques. For example, such fragments include but are not limited to the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$

fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

The generation of anti-TGF-β antibodies is described in copending application Serial No. 7/353,728 filed August 17, 1989, and in copending application Serial No. 07/446,020 filed December 5, 1989.

5.2.2. TGF-β RECEPTORS

Exogenous TGF-β receptor molecules may be useful pressor agents inasmuch as they are capable of binding circulating TGF-β and/or out-competing endogenous receptors which may initiate the hypotensive effect of TGF-β. TGF-β receptors may be prepared by the methods described in copending application Serial No. 269,524 filed November 11, 1988, which application is incorporated by reference herein in its entirety.

6. EXAMPLE: EFFECT OF PARENTERALLY ADMINISTERED rTGF-β1 ON BLOOD PRESSURE IN CYNOMOLGUS MONKEYS

Described below is part of a study designed to evaluate the pharmacotoxic effects of rTGF-β1 following daily intravenous infusions to cynomolgus monkeys (Macaca fascicularis). The results described herein indicate that rTGF-β1 has a profound reducing effect on blood pressure.

6.1. PROTOCOL

6.1.1. CYNOMOLGUS MONKEYS

One adult male and two adult female cynomolgus monkeys were monitored for blood pressure changes resulting from daily TGF-β1 treatment. Monkeys were fed commerically available chow daily and were provided water ad libitum. Blood pressures were measured via chronic arterial catheters surgically implanted at least 5 days prior to the initiation of the study. One of the female monkeys (39-181) underwent

general anesthesia and a chronic venous catheter was implanted in the right illiac vein. Approximately one month later, this catheter was removed, and the monkey was asepically implanted with chronic arterial and venous catheters in the illiac artery and vein. The other female monkey (29-825) and the male monkey (B-344) were also implanted with chronic venous and arterial catheters in the illiac artery and vein. The catheters were exteriorized via a tether apparatus as a venous access to facillitate the administration of the test article or vehicle control.

6.1.2. TEST ARTICLE FORMULATION

The formulation of the test and/or control articles was performed daily prior to administration. Each 10 ml of test article dosing solution (0.0213 mg/ml) was prepared by mixing 0.66 ml of rTGF-β1 stock solution (0.46 mg/ml in 5mM HCl) with 9.34 ml of 0.1% monkey serum albumin in PBS solution. The pH of the dosing solution was recorded after formulation and prior to administration each day. Dose volumes were calculated based on the most recent non-tethered body weights and rounded to the nearest 0.1 ml.

Recombinant mature TGF-β1 was isolated and purified from the supernatants of cultured Chinese Hamster Ovary cells transfected with the complete simian TGF-β1 precursor coding sequence as described in co-pending application Serial No. 07/353,728 filed August 17, 1989, which application is incorporated by reference herein in its entirety.

6.1.3. TREATMENT

Monkey 39-181 received 1% monkey albumin in PBS (vehicle control) at a volume of 8 ml/kg daily for five consecutive days. Monkey 69-168 was treated for five consecutive days at a dose of 0.17 mg/kg and at a

concentration of 0.0213 mg/ml. rTGF-β1 was administered to Monkeys 29-825, 39-181 and B344 at a dose of 0.51 mg/kg and at a concentration of 0.0639 mg/ml. Monkey 29-825 was treated for three consecutive days, monkey 39-181 for three consecutive days, and monkey B-344 for one day. The body weights used to calculate the dosages of either the test article or control were the most recent body weights obtained without the encumbrance of the tether apparatus.

rTGF-β1 or vehicle control was administered intravenously through chronic venous catheters at a rate of 1.60 ml of dosing solution/minute via an infusion pump (Harvard Apparatus), and calibrated according to the standard operating procedures of the test facility. Prior to the administration of the test article and control, catheter patency was maintained via periodic flushing of the catheter with 0.9% sterile saline. The volume, time, and date of administration of rTGF-β1 and control were recorded.

6.1.4. BLOOD PRESSURE MEASUREMENTS

Blood pressure measurements were recorded from monkeys 29-825, 39-181, and B344 via chronic arterial catheters. Blood pressure measurements were recorded for at least one minute prior to and after completion of the administration of the test article. Blood pressure measurements were also recorded as amended or at the discretion of the study director if such measurements were clinically relevant.

6.2. CLINICAL OBSERVATIONS

The monkeys were observed daily over 29 days for clinical abnormalities, food and water intake, body temperature, respiration rate, blood pressure, and other

parameters. Additionally, blood samples were collected from each monkey to provide samples for hematology, serum chemistry and immunological analyses.

6.2.1. EFFECT OF rTGF-β1 ON BP

Two of the three monkeys receiving rTGF-β1 injections experienced immediate, significant and progressive decreases in arterial blood pressure. The third experimental monkey also experienced BP loss, but these results are somewhat more difficult to interpret in view of the extreme hypotension existing in this animal prior to treatment. No significant BP fluctuations were observed in the control monkey. The individual BP observations for the three experimental monkeys are tabulated in TABLE I.

TABLE I

INDIVIDUAL BLOOD PRESSURE PROFILES OF MALE AND FEMALE CYNOMOLGUS MONKEYS TREATED WITH TGF-β1

ANIMAL NUMBER	SEX	DOSE mg/kg	STUDY DAY	MEAL ATERIAL BLOOD PRESSURE (mm Hg)
39-181	F	0.51	1 (Pre)	44
			1 (Post)	48
			1 (4 Hr)	50
			2 (Pre)	60
			2 (Post)	32
			2 (4 Hr)	46
			3 (Pre)	40
			3 (Post)	32
			3 (4 Hr)	12
			4	18
			5	32
29-825	F	0.51	1 (Pre)	108
			1 (Post)	80
			1 (4 Hr)	48
			2 (Pre)	76
			2 (Post)	76
			3 (Pre)	20
			4	52
			5	50
B-344	M	0.51	1 (Pre)	104
			1 (Post)	60
			1 (4 Hr)	36
			2	20
			3	32
			4	22
			5	20
			9	36

Monkeys 29-825 and B-344 experienced an immediate (1 hour post-administration) BP reduction of 26% and 42%, respectively. Four hours after treatment, BP had dropped by 55% in monkey 29-825 and by 65% in monkey B-344. These initial drops in BP were sustained in the subsequent treatment days, resulting in hypotension and shock. Monkey 39-181 did not respond to initial TGF-β1 treatment (day 1) with a reduction in BP, possibly because of its pre-existing hypotensive condition. Interestingly, a slight elevation in BP was observed on day 2 prior to treament, and a sustained decrease in BP was observed thereafter.

In addition to the dramatic decrease in BP and the accompanying shock/hypotension observed in all three treated animals, other observed effects directly attributable to TGF-β1 included hemotopoietic changes (decrease in erythrocytes, lymphocytes and platlets) and immunological compromise (decrease in lymphocyte responsiveness to mitogen). Additionally, all the treated monkeys had no or minimal appetite, and all were inactive during the treatment period. Monkey 29-825 was recumbent on day 4, required fluid therapy on days 4 and 5, and was euthanized on day 5 because of its moribund condition. Monkey 39-181 had darkened blood on days 3 and 4, developed septicemia on day 8, and was euthanized because of its deteriorating condition on day 8. Monkey B-344 appeared normal from days 6 to 29.

The present invention is not to be limited in scope by the cell lines, TGF-β molecules and assays exemplified which are intended as but single illustrations of one aspect of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled

in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating hypertension comprising administering a TGF-β to an individual at a dose effective at lowering blood pressure.

2. The method of claim 1 wherein the TGF-β comprises a mature TGF-β1.

3. The method of claim 1 wherein the TGF-β comprises a mature TGF-β2.

4. The method of claim 1 wherein the TGF-β comprises a mature TGF-β1/β2 hybrid.

5. The method of claim 1 wherein the TGF-β comprises a TGF-β1 precursor.

6. The method of claim 1 wherein the TGF-β comprises a TGF-β2 precursor.

7. The method of claim 1 wherein the TGF-β comprises a hybrid TGF-β1/TGF-β2 precursor.

8. The method of claim 1 wherein the TGF-β comprises a latent TGF-β1 complex.

9. The method of claim 1 wherein the TGF-β comprises a latent TGF-β2 complex.

10. A method of lowering blood pressure in a mammal comprising administering TGF-β to the mammal in an amount and for a time period effective at inducing the desired hypotensive effect.

11. The method of claim 10 wherein the TGF-β comprises a mature TGF-β1.

12. The method of claim 10 wherein the TGF-β comprises a mature TGF-β2.

13. The method of claim 10 wherein the TGF-β comprises a mature TGF-β1/β2 hybrid.

14. The method of claim 10 wherein the TGF-β comprises a TGF-β1 precursor.

15. The method of claim 10 where in the TGF-β comprises a TGF-β2 precursor.

16. The method of claim 10 where in the TGF-β comprises a hybrid TGF-β1/TGF-β2 precursor.

17. The method of claim 10 where in the TGF-β comprises a latent TGF-β1 complex.

18. The method of claim 10 where in the TGF-β comprises a latent TGF-β2 complex.

19. A method of treating hypotension comprising administering to an individual a TGF-β antagonist capable of inhibiting the hypotensive activity induced by TGF-β at a dose effective at inducing an elevation in blood pressure.

20. The method of claim 19 wherein the TGF-β antagonist is an anti-TGF-β antibody.

21. The method of claim 19 wherein the TGF-β antagonist is a TGF-β receptor.

22. The method of claim 19 wherein the TGF-β antagonist is TGF-β1 antagonist.

23. The method of claim 22 wherein the TGF-β1 antagonist is an anti-TGF-β1 antibody.

24. The method of claim 22 wherein the TGF-β antagonist is a TGF-β1 receptor.

25. The method of claim 19 wherein the TGF-β antagonist is a TGF-β2 antagonist.

26. The method of claim 25 wherein the TGF-β2 antagonist is a TGF-β2 antibody.

27. The method of claim 25 wherein the TGF-β2 antagonist is a TGF-β2 receptor.

28. A method of elevating blood pressure comprising administering to a mammal a TGF-β antagonist in an amount and for a time period effective at inducing the desired blood pressure increase.

29. The method of claim 28 wherein the TGF-β antagonist is an anti-TGF-β antibody.

30. The method of claim 28 wherein the TGF-β antagonist is a TGF-β receptor.

31. The method of 28 wherein the TGF-β antagonist is a TGF-β1 antagonist.

32. The method of claim 31 wherein the TGF-β1 antagonist is an anti-TGF-β1 antibody.

33. The method of claim 31 wherein the TGF-β1 antagonist is a TGF-β1 receptor.

34. The method of claim 28 wherein the TGF-β antagonist is a TGF-β2 antagonist.

35. The method of claim 34 wherein the TGF-β2 antagonist is a TGF-β2 antibody.

36. The method of claim 34 wherein the TGF-β2 antagonist is a TGF-β2 receptor.

```
Simian      -261  AGGGGATCTGTGGCAGGTCGGAGA---AAGATC---CGTCT -227
Human             CTCC..C...CAA...A..CCCT.TTC....C.ACC.AC..

Simian CCTGGTACCAGATCTCGCCCATCTAGGTTATTTCCGTGGGATACTGAGACAC -175
Human  T.............G.....................................

Simian CCCCGGTCCAAGCCTCCCCTCCACCACTGCGCCCTTCTCCCGTAGGA-CCTC -123
Human  ..........................................G.....

Simian AACTTTCCCTCGAGGCCCTCCTACCTTTTCCCGGGGGACCCCCAGCCCCTGC  -71
Human  G.........................G.....A................

Simian AGGGGCGGGGCCTCCCCACCAAACTAGCCCTGTTCGCGCTCTCGGCAGTGCC  -19
Human  ......................C..C.........................

                                Met Pro Pro Ser Gly Leu Arg Leu
Simian GGGGGGCGCCGCCTCCCC ATG CCG CCC TCC GGG CTG CGG CTG     24
Human  .................. ... ... ... ... ... ... ... ...

       10                                                20
       Leu Pro Leu Leu Leu Pro Leu Leu Trp Leu Leu Val Leu
Simian CTG CCG CTG CTG CTA CCG CTG CTG TGG CTA CTG GTG CTG     63
Human  ... ... ... ... ... ... ... ... ... ... ... ... ...

          Gly Pro                    30
       Thr Pro Ser Arg Pro Ala Ala Gly Leu Ser Thr Cys Lys
Simian ACG CCT AGC CGG CCG GCC GCA GGA CTA TCC ACC TGC AAG    102
Human  ... ... G.. .C. ... ... ... ... ... ... ... ... ...

                         40
       Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu
Simian ACT ATC GAC ATG GAG CTG GTG AAG CGG AAG CGC ATC GAG    141
Human  ... ... ... ... ... ... ... ... ... ... ... ... ...

       Ala      50                                        60
       Thr Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala
Simian ACC ATC CGC GGC CAG ATC CTG TCC AAG CTG CGG CTC GCC    180
Human  G.. ... ... ... ... ... ... ... ... ... ... ... ...

                                  70
       Ser Pro Pro Ser Gln Gly Glu Val Pro Pro Gly Pro Leu
Simian AGC CCC CCG AGC CAG GGG GAG GTG CCG CCC GGC CCG CTG    219
Human  ... ... ... ... ... ... ... ... ... ... ... ... ...

                         80
       Pro Glu Ala Val Leu Ala Leu Tyr Asn Ser Thr Arg Asp
Simian CCC GAG GCC GTG CTC GCC CTG TAC AAC AGC ACC CGC GAC    258
Human  ... ... ... ... ... ... ... ... ... ... ... ... ...
```

FIG. 1

```
                                90
          Arg Val Ala Gly Glu Ser Ala Glu Pro Glu Pro Glu Pro
Simian    CGG GTG GCC GGG GAG AGT GCG GAG CCG GAG CCC GAA CCG   297
Human     ... ... ... ... ... ... ..A ..A ... ... ... ..G ..T

          100                                             110
          Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu   336
Simian    GAG GCC GAC TAC TAC GCC AAG GAG GTC ACC CGC GTG CTA
Human     ... ... ... ... ... ... ... ... ... ... ... ... ...

                                        120
          Met Val Glu Thr His Asn Glu Ile Tyr Asp Lys Phe Lys
Simian    ATG GTG GAA ACC CAC AAC GAA ATC TAT GAC AAG TTC AAG   375
Human     ... ... ... ... ... ... ... ... ... ... ... ... ...

                    130
          Gln Ser Thr His Ser Ile Tyr Met Phe Phe Asn Thr Ser
Simian    CAG AGC ACA CAC AGC ATA TAT ATG TTC TTC AAC ACA TCA   414
Human     ... ..T ... ... ... ... ... ... ... ... ... ... ...

                                                    150
          Glu 140                                   Leu Ser
          Glu Leu Arg Glu Ala Val Pro Glu Pro Val Leu Leu Ser
Simian    GAG CTC CGA GAA GCA GTA CCT GAA CCT GTG TTG CTC TCC   453
Human     ... ... ... ... ..G ... ... ... ..C ... ... ... ...

                                        Arg     160
          Arg Ala Glu Leu Arg Leu Leu   --- Arg Leu Lys Leu Lys
Simian    CGG GCA GAG CTG CGT CTG CTG   --- AGG CTC AAG TTA AAA   492
Human     ... ... ... ... ... ... ... AGG ... ... ... ... ...

                                170
          Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn
Simian    GTC GAG CAG CAT GTG GAG CTG TAC CAG AAA TAC AGC AAC   531
Human     ... ... ... ..C ... ... ... ... ... ... ... ... ...

                    180
          Asn Ser Trp Arg Tyr Leu Ser Asn Arg Leu Leu Ala Pro
Simian    AAT TCC TGG CGA TAC CTC AGC AAC CGG CTG CTG GCG CCC   570
Human     ... ... ... ... ... ... ... ... ... ... ..A ... ...

          190 Asp                                         200
          Ser Asn Ser Pro Glu Trp Leu Ser Phe Asp Val Thr Gly
Simian    AGC AAC TCG CCG GAG TGG TTG TCT TTT GAT GTC ACC GGA   609
Human     ... G.. ... ..A ... ... ..A ... ... ... ... ... ...

                                            210
          Val Val Arg Gln Trp Leu Ser Arg Gly Gly Glu Ile Glu
Simian    GTT GTG CGG CAG TGG TTG AGC CGC GGA GGG GAA ATT GAG   648
Human     ... ... ... ... ... ... ... ..T ... ... ... ... ...
```

FIG. 1(cont.)

```
                                220                                 Arg
        Gly Phe Arg Leu Ser Ala His Cys Ser Cys Asp Ser Lys
Simian  GGC TTT CGC CTT AGC GCC CAC TGC TCC TGT GAC AGC AAA   687
Human   ... ... ... ... ... ... ... ... ... ... ... .GG

            230                                 240
        Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe Thr Thr
Simian  GAT AAC ACA CTG CAA GTG GAC ATC AAC GGG TTC ACT ACC   726
Human   ... ... ... ... ... ... ... ... ... ... ... ... ...

                                    250
        Gly Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn
Simian  GGC CGC CGA GGT GAC CTG GCC ACA ATT CAT GGC ATG AAC   765
Human   ... ... ... ... ... ... ... ..C ... ... ... ... ...

                            260
        Arg Pro Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg
Simian  CGG CCT TTC CTG CTT CTC ATG GCC ACC CCG CTG GAG AGG   804
Human   ... ... ... ... ... ... ... ... ... ... ... ... ...

                270                                         280
        Ala Gln His Leu Gln Ser Ser Arg His Arg Arg Ala Leu
Simian  GCC CAA CAT CTG CAA AGC TCC CGG CAC CGC CGA GCC CTG   843
Human   ... ..G ... ... ... ... ... ... ... ... ... ... ...

                                            290
        Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys Asn Cys
Simian  GAC ACC AAC TAC TGC TTC AGC TCC ACG GAG AAG AAC TGC   882
Human   ... ... ... ..T ... ... ... ... ... ... ... ... ...

                                300
        Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu
Simian  TGC GTG CGG CAG CTG TAT ATT GAC TTC CGC AAG GAC CTC   921
Human   ... ... ... ... ... ..C ... ... ... ... ... ... ...

                    310
        Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala
Simian  GGC TGG AAG TGG ATC CAC GAG CCC AAG GGC TAC CAT GCC   960
Human   ... ... ... ... ... ... ... ... ... ... ... ... ...

        320                                         330
        Asn Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu
Simian  AAC TTC TGC CTG GGG CCC TGT CCC TAC ATT TGG AGC CTG   999
Human   ... ... ... ..C ... ... ..C ... ... ... ... ... ...

                                    340
        Asp Thr Gln Tyr Ser Lys Val Leu Ala Leu Tyr Asn Gln
Simian  GAC ACG CAG TAC AGC AAG GTC CTG GCC CTG TAC AAC CAG  1038
Human   ... ... ... ... ... ... ... ... ... ... ... ... ...
```

FIG. 1(cont.)

```
                          350
          His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys Val Pro
Simian    CAT AAC CCG GGC GCC TCG GCG GCG CCG TGC TGC GTG CCG  1077
Human     ... ... ... ... ... ... ... ... ... ... ... ... ...

     360                                                  370
          Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly
Simian    CAG GCG CTG GAG CCA CTG CCC ATC GTG TAC TAC GTG GGC  1116
Human     ... ... ... ... ..G ... ... ... ... ... ... ... ...

                                    380
          Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val
Simian    CGC AAG CCC AAG GTG GAG CAG CTG TCC AAC ATG ATC GTG  1155
Human     ... ... ... ... ... ... ... ... ... ... ... ... ...

                          390
          Arg Ser Cys Lys Cys Ser
Simian    CGC TCC TGC AAA TGC AGC TGA GGCCCCGCCCCGCCCCGCCCCAC  1199
Human     ... ... ... ..G ... ... ... ..T.................G.

Simian    CCCGGCAGGCCCGGCCCCGCCCCACCCCACCCCCGCTGTCTTGCCCTTGGG  1250
Human     ..................A....G....G.........C.......A....

Simian    GGCTGTATTTAAGGACACCCGTGCCCCAAGCCCACCTGGGGCCCCATTAAA
Human     ..................................................

Simian    GA                                                   1303
Human     ..
```

FIG. 1(cont.)

```
GCCCCTCCCGTCAGTTCGCCAGCTGCCAGCCCCGGGACCTTTTCATCTCTTCCCTTTG   -409

GCCGGAGGAGCCGAGTTCAGATCCGCCACTCCGCACCCGAGACTGACACACTGAACTC   -351

CACTTCCTCCTCTTAAATTTATTTCTACTTAATAGCCACTCGTCTCTTTTTTTCCCCA   -293

TCTCATTGCTCCAAGAATTTTTTTCTTCTTACTCGCCAAAGTCAGGGTTCCCTCTGCC   -235

CGTCCCGTATTAATATTTCCACTTTTGGAACTACTGGCCTTTTCTTTTTAAAGGAATT   -177

CAAGCAGGATACGTTTTTCTGTTGGGCATTGACTAGATTGTTTGCAAAAGTTTCGCAT   -119

CAAAAACAACAACAACAAAAAACCAAACAACTCTCCTTGATCTATACTTTGAGAATTG    -61

TTGATTTCTTTTTTTTATTCTGACTTTTAAAAACAACTTTTTTTTCCACTTTTTTAAA     -3

     1                                        10
   Met His Tyr Cys Val Leu Ser Ala Phe Leu Ile Leu His Leu
AA ATG CAC TAC TGT GTG CTG AGC GCT TTT CTG ATC CTG CAT CTG      42
                                        T

                        20
Val Thr Val Ala Leu Ser Leu Ser Thr Cys Ser Thr Leu Asp Met
GTC ACG GTC GCG CTC AGC CTG TCT ACC TGC AGC ACA CTC GAT ATG     87

 30                                      40
Asp Gln Phe Met Arg Lys Arg Ile Glu Ala Ile Arg Gly Gln Ile
GAC CAG TTC ATG CGC AAG AGG ATC GAG GCG ATC CGC GGG CAG ATC    132

                    50
Leu Ser Lys Leu Lys Leu Thr Ser Pro Pro Glu Asp Tyr Pro Glu
CTG AGC AAG CTG AAG CTC ACC AGT CCC CCA GAA GAC TAT CCT GAG    177

 60                                      70       *
Pro Glu Glu Val Pro Pro Glu Val Ile Ser Ile Tyr Asn Ser Thr
CCC GAG GAA GTC CCC CCG GAG GTG ATT TCC ATC TAC AAC AGC ACC    222

                    80
Arg Asp Leu Leu Gln Glu Lys Ala Ser Arg Arg Ala Ala Ala Cys
AGG GAC TTG CTC CAG GAG AAG GCG AGC CGG AGG GCG GCC GCC TGC    267

 90                                     100
Glu Arg Glu Arg Ser Asp Glu Glu Tyr Tyr Ala Lys Glu Val Tyr
GAG CGC GAG AGG AGC GAC GAA GAG TAC TAC GCC AAG GAG GTT TAC    312

                   110
Lys Ile Asp Met Pro Pro Phe Phe Pro Ser Glu Thr Val Cys Pro
AAA ATA GAC ATG CCG CCC TTC TTC CCC TCC GAA ACT GTC TGC CCA    357

120                                     130
Val Val Thr Thr Pro Ser Gly Ser Val Gly Ser Leu Cys Ser Arg
GTT GTT ACA ACA CCC TCT GGC TCA GTG GGC AGC TTG TGC TCC AGA    402
```

FIG. 2

```
                          140
Gln Ser Gln Val Leu Cys Gly Tyr Leu Asp | Ala Ile Pro Pro Thr
CAG TCC CAG GTG CTC TGT GGG TAC CTT GAT | GCC ATC CCG CCC ACT    447

150                                                 160
Phe Tyr Arg Pro Tyr Phe Arg Ile Val Arg Phe Asp Val Ser Ala
TTC TAC AGA CCC TAC TTC AGA ATT GTT CGA TTT GAC GTC TCA GCA    492
                                        G

                *         170
Met Glu Lys Asn Ala Ser Asn Leu Val Lys Ala Glu Phe Arg Val
ATG GAG AAG AAT GCT TCC AAT TTG GTG AAA GCA GAG TTC AGA GTC    537

180                                                 190
Phe Arg Leu Gln Asn Pro Lys Ala Arg Val Pro Glu Gln Arg Ile
TTT CGT TTG CAG AAC CCA AAA GCC AGA GTG CCT GAA CAA CGG ATT    582
                  T

                          200
Glu Leu Tyr Gln Ile Leu Lys Ser Lys Asp Leu Thr Ser Pro Thr
GAG CTA TAT CAG ATT CTC AAG TCC AAA GAT TTA ACA TCT CCA ACC    627
   G                                    C

210                                                 220
Gln Arg Tyr Ile Asp Ser Lys Val Val Lys Thr Arg Ala Glu Gly
CAG CGC TAC ATC GAC AGC AAA GTT GTG AAA ACA AGA GCA GAA GGC    672

                          230
Glu Trp Leu Ser Phe Asp Val Thr Asp Ala Val His Glu Trp Leu
GAA TGG CTC TCC TTC GAT GTA ACT GAT GCT GTT CAT GAA TGG CTT    717
         T

240                                                 250
His His Lys Asp Arg Asn Leu Gly Phe Lys Ile Ser Leu His Cys
CAC CAT AAA GAC AGG AAC CTG GGA TTT AAA ATA AGC TTA CAC TGT    762

                                                          *
                          260
Pro Cys Cys Thr Phe Val Pro Ser Asn Asn Tyr Ile Ile Pro Asn
CCC TGC TGC ACT TTT GTA CCA TCT AAT AAT TAC ATC ATC CCA AAT    807

270                                                 280
Lys Ser Glu Glu Leu Glu Ala Arg Phe Ala Gly Ile Asp Gly Thr
AAA AGT GAA GAA CTA GAA GCA AGA TTT GCA GGT ATT GAT GGC ACC    852
                  T

                          290
Ser Thr Tyr Thr Ser Gly Asp Gln Lys Thr Ile Lys Ser Thr Arg
TCC ACA TAT ACC AGT GGT GAT CAG AAA ACT ATA AAG TCC ACT AGG    897

300                                                 310
Lys Lys Asn Ser Gly Lys Thr Pro His Leu Leu Leu Met Leu Leu
AAA AAA AAC AGT GGG AAG ACC CCA CAT CTC CTG CTA ATG TTA TTG    942
```

FIG. 2(cont.)

```
                          320
Pro Ser Tyr Arg Leu Glu Ser Gln Gln Thr Asn Arg Arg Lys Lys
CCC TCC TAC AGA CTT GAG TCA CAA CAG ACC AAC CGG CGG AAG AAG   987

330                                                   340
Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn
CGT GCT TTG GAT GCG GCC TAT TGC TTT AGA AAT GTG CAG GAT AAT  1032

                    350
Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly
TGC TGC CTA CGT CCA CTT TAC ATT GAT TTC AAG AGG GAT CTA GGG  1077
                  G

360                                                   370
Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys
TGG AAA TGG ATA CAC GAA CCC AAA GGG TAC AAT GCC AAC TTC TGT  1122
                                  A

                    380
Ala Gly Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser
GCT GGA GCA TGC CCG TAT TTA TGG AGT TCA GAC ACT CAG CAC AGC  1167

390                                                   400
Arg Val Leu Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala
AGG GTC CTG AGC TTA TAT AAT ACC ATA AAT CCA GAA GCA TCT GCT  1212

                    410
Ser Pro Cys Cys Val Ser Gln Asp Leu Glu Pro Leu Thr Ile Leu
TCT CCT TGC TGC GTG TCC CAA GAT TTA GAA CCT CTA ACC ATT CTC  1257
                              C

420                                                   430
Tyr Tyr Ile Gly Lys Thr Pro Lys Ile Glu Gln Leu Ser Asn Met
TAC TAC ATT GGC AAA ACA CCC AAG ATT GAA CAG CTT TCT AAT ATG  1302

                    440
Ile Val Lys Ser Cys Lys Cys Ser
ATT GTA AAG TCT TGC AAA TGC AGC TAA AATTCTTGGAAAAGTGGCAAGA  1351

CCAAAATGACAATGATGATGATAATGATGATGACGACGACAACGATGATGCTTGTAAC  1409

AAGAAAACATAAGAGAGCCTTGGTTCATCAGTGTTAAAAAATTTTTGAAAAGGCGGTA  1467

CTAGTTCAGACACTTTGGAAGTTTGTGTTCTGTTTGTTAAAACTGGCATCTGACACAA  1525

AAAAAGTTGAAGGCCTTATTCTACATTTCACCTACTTTGTAAGTGAGAGAGACAAGAA  1583
```

FIG. 2(cont.)

GCAAATTTTTTTTAAAGAAAAAAATAAACACTGGAAGAATTTATTAGTGTTAATTATG 1641

TGAACAACGACAACAACAACAACAACAACAAACAGGAAAATCCCATTAAGTGGAGTTG 1699

CTGTACGTACCGTTCCTATCCCGCGCCTCACTTGATTTTTCTGTATTGCTATGCAATA 1757

GGCACCCTTCCCATTCTTACTCTTAGAGTTAACAGTGAGTTATTTATTGTGTGTTACT 1815

ATATAATGAACGTTTCATTGCCCTTGGAAAATAAAACAGGTGTATAAAGTGGAGACCA 1873

AATACTTTGCCAGAAACTCATGGATGGCTTAAGGAACTTGAACTCAAACGAGCCAGAA 1931

AAAAAGAGGTCATATTAATGGGATGAAAACCCAAGTGAGTTATTATATGACCGAGAAA 1989

GTCTGCATTAAGATAAAGACCCTGAAAACACATGTTATGTATCAGCTGCCTAAGGAAG 2047

CTTCTTGTAAGGTCCAAAAACTAAAAAGACTGTTAATAAAAGAAACTTTCAGT 2100

CAG(poly A) 2103

FIG. 2(cont.)

```
-261 AGGGGATCTGTGGCAGGTCGGAGA---AAGATC---CGTCTCCTGGTACCAG   -215

ATCTCGCCCATCTAGGTTATTTCCGTGGGATACTGAGACACCCCCGGTCCAAGCCTCC   -157

CCTCCACCACTGCGCCCTTCTCCCGTAGGA-CCTCAACTTTCCCTCGAGGCCCTCCTA   -101

CCTTTTCCCGGGGGACCCCCAGCCCCTGCAGGGGCGGGGCCTCCCCACCAAACTAGCC   -43

                                                      1
                                                  Met Pro Pro Ser
CTGTTCGCGCTCTCGGCAGTGCCGGGGGGCGCCGCCTCCCCC ATG CCG CCC TCC    12

                    10
Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu Leu Trp Leu Leu
GGG CTG CGG CTG CTG CCG CTG CTG CTA CCG CTG CTG TGG CTA CTG    57

 20                                                  30
Val Leu Thr Pro Ser Arg Pro Ala Ala Gly Leu Ser Thr Cys Lys
GTG CTG ACG CCT AGC CGG CCG GCC GCA GGA CTA TCC ACC TGC AAG   102

                        40
Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Thr Ile
ACT ATC GAC ATG GAG CTG GTG AAG CGG AAG CGC ATC GAG ACC ATC   147

 50                                                  60
Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala Ser Pro Pro Ser
CGC GGC CAG ATC CTG TCC AAG CTG CGG CTC GCC AGC CCC CCG AGC   192

                        70
Gln Gly Glu Val Pro Pro Gly Pro Leu Pro Glu Ala Val Leu Ala
CAG GGG GAG GTG CCG CCC GGC CCG CTG CCC GAG GCC GTG CTC GCC   237

 80                                                  90
Leu Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Ala Glu
CTG TAC AAC AGC ACC CGC GAC CGG GTG GCC GGG GAG AGT GCG GAG   282

                       100
Pro Glu Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr
CCG GAG CCC GAA CCG GAG GCC GAC TAC TAC GCC AAG GAG GTC ACC   327

110                                                 120
Arg Val Leu Met Val Glu Thr His Asn Glu Ile Tyr Asp Lys Phe
CGC GTG CTA ATG GTG GAA ACC CAC AAC GAA ATC TAT GAC AAG TTC   372

                       130
Lys Gln Ser Thr His Ser Ile Tyr Met Phe Phe Asn Thr Ser Glu
AAG CAG AGC ACA CAC AGC ATA TAT ATG TTC TTC AAC ACA TCA GAG   417

140                                                 150
Leu Arg Glu Ala Val Pro Glu Pro Val Leu Leu Ser Arg Ala Glu
CTC CGA GAA GCA GTA CCT GAA CCT GTG TTG CTC TCC CGG GCA GAG   462
```

FIG. 3

```
                                160
Leu Arg Leu Leu                 Arg Leu Lys Leu Lys Val Glu Gln His Val
CTG CGT CTG CTG ___             AGG CTC AAG TTA AAA GTC GAG CAG CAT GTG    504

170                                                 180
Glu Leu Tyr Gln Lys Tyr Ser Asn Asn Ser Trp Arg Tyr Leu Ser
GAG CTG TAC CAG AAA TAC AGC AAC AAT TCC TGG CGA TAC CTC AGC    549

                                190
Asn Arg Leu Leu Ala Pro Ser Asn Ser Pro Glu Trp Leu Ser Phe
AAC CGG CTG CTG GCG CCC AGC AAC TCG CCG GAG TGG TTG TCT TTT    594

200                                                 210
Asp Val Thr Gly Val Val Arg Gln Trp Leu Ser Arg Gly Gly Glu
GAT GTC ACC GGA GTT GTG CGG CAG TGG TTG AGC CGC GGA GGG GAA    639

                                220
Ile Glu Gly Phe Arg Leu Ser Ala His Cys Ser Cys Asp Ser Lys
ATT GAG GGC TTT CGC CTT AGC GCC CAC TGC TCC TGT GAC AGC AAA    684

230                                                 240
Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe Thr Thr Gly Arg
GAT AAC ACA CTG CAA GTG GAC ATC AAC GGG TTC ACT ACC GGC CGC    729

                                250
Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg Pro Phe Leu
CGA GGT GAC CTG GCC ACA ATT CAT GGC ATG AAC CGG CCT TTC CTG    774

260                                                 270
Leu Leu Met His Thr Pro Leu Glu Arg Ala Gln His Leu Gln Ser
CTT CTC ATG GCC ACC CCG CTG GAG AGG GCC CAA CAT CTG CAA AGC    819

                                280
Ser Arg His Arg Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn
TCC CGG CAC CGC CGA GCT TTG GAT GCG GCC TAT TGC TTT AGA AAT    864

290                                                 300
Val Gln Asp Asn Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys
GTG CAG GAT AAT TGC TGC CTA CGT CCA CTT TAC ATT GAT TTC AAG    909
                                  G

                                310
Arg Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Asn
AGG GAT CTA GGG TGG AAA TGG ATA CAC GAA CCC AAA GGG TAC AAT    954
                                                    A

320                                                 330
Ala Asn Phe Cys Ala Gly Ala Cys Pro Tyr Leu Trp Ser Ser Asp
GCC AAC TTC TGT GCT GGA GCA TGC CCG TAT TTA TGG AGT TCA GAC    999

                                340
Thr Gln His Ser Arg Val Leu Ser Leu Tyr Asn Thr Ile Asn Pro
ACT CAG CAC AGC AGG GTC CTG AGC TTA TAT AAT ACC ATA AAT CCA   1044
```

FIG. 3(cont.)

SUBSTITUTE SHEET

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350                                                  360
Glu Ala Ser Ala Ser Pro Cys Cys Val Ser Gln Asp Leu Glu Pro
GAA GCA TCT GCT TCT CCT TGC TGC GTG TCC CAA GAT TTA GAA CCT  1089
                                      C

                    370
Leu Thr Ile Leu Tyr Tyr Ile Gly Lys Thr Pro Lys Ile Glu Gln
CTA ACC ATT CTC TAC TAC ATT GGC AAA ACA CCC AAG ATT GAA CAG  1134

380                                                  390
Leu Ser Asn Met Ile Val Lys Ser Cys Lys Cys Ser ***
CTT TCT AAT ATG ATT GTA AAG TCT TGC AAA TGC AGC TAA AATTCT  1179

TGGAAAAGTGGCAAGACCAAAATGACAATGATGATGATAATGATGATGACGACGACAA  1237

CGATGATGCTTGTAACAAGAAAACATAAGAGAGCCTTGGTTCATCAGTGTTAAAAAAT  1295

TTTTGAAAAGGCGGTACTAGTTCAGACACTTTGGAAGTTTGTGTTCTGTTTGTTAAAA  1353

CTGGCATCTGACACAAAAAAAGTTGAAGGCCTTATTCTACATTTCACCTACTTTGTAA  1411

GTGAGAGAGACAAGAAGCAAATTTTTTTTAAAGAAAAAAATAAACACTGGAAGAATTT  1469

ATTAGTGTTAATTATGTGAACAACGACAACAACAACAACAACAAACAGGAAAATC  1527

CCATTAAGTGGAGTTGCTGTACGTACCGTTCCTATCCCGCGCCTCACTTGATTTTTCT  1585

GTATTGCTATGCAATAGGCACCCTTCCCATTCTTACTCTTAGAGTTAACAGTGAGTTA  1643

TTTATTGTGTGTTACTATATAATGAACGTTTCATTGCCCTTGGAAAATAAAACAGGTG  1701

TATAAAGTGGAGACCAAATACTTTGCCAGAAACTCATGGATGGCTTAAGGAACTTGAA  1759

CTCAAACGAGCCAGAAAAAAAGAGGTCATATTAATGGGATGAAAACCCAAGTGAGTTA  1817

TTATATGACCGAGAAAGTCTGCATTAAGATAAAGACCCTGAAAACACATGTTATGTAT  1875

CAGCTGCCTAAGGAAGCTTCTTGTAAGGTCCAAAAACTAAAAAGACTGTTAATAAAAG  1933

AAACTTTCAGTCAG(poly A)                                       1947
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FIG. 3(cont.)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/04449

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/36
US.CL.: 514/8

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	514/2,8,12,21; 424/85.1, 88; 530/380,395,399

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Databases: Dialog (Files 5, 73, 155, 351); USPTO Automated Patent System (File USPAT, 1971-1991).

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	NATURE, Vol. 316, issued 22 August 1985, Derynck et al., "Human transforming Growth Factor-β Complementary DNA Sequence and Expression in Normal and Transformed Cells," pages 701-705, see entire document.	1-2,4-5, 7-8,10-11 13-14,16-17
Y	Cell, Vol. 49, issued 22 May 1987, Massague, TGF-B Family of Growth and Differentiation Factors," pages 437-438, see entire document.	1-2,4-11, 13-18
Y	DNA, Vol. 7, NO. 1, issued 1988, Madisen et al., "Transforming Growth Factor-B2: cDNA Cloning and Sequence Analysis," pages 1-8, see entire document.	1,4,6,7, 9-10,13 15-16,18.
Y	DNA, Vol. 7, No. 7, issued 1988, Webber et al., "Structural and Sequence Analysis of TGF-B2 cDNA Clones Predicts Two Different Precursor Proteins Produced by Alternative mRNA splicing," pages 493-497, see entire document.	1,6,7,10 15,16,18

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 September 1991	27 SEP 1991
International Searching Authority	Signature of Authorized Officer
ISA/US	Robert D. Budens tf

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Cell, Vol. 48, issued 13 February 1987, Cheifetz et al., "The Transforming Growth Factor-B System, a Complex Pattern of Cross-Reactive Ligands and Receptores", pages 409-415, see entire document.	1,4,7,10, 13,16

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

SEE ATTACHED SHEET

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims: 1-2,4-11,13-18

TELEPHONE PRACTICE

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

In the examination of international applications filed under the Patent Cooperation Treaty, PCT Rule 13.1 states that the international application shall relate to one invention only or to a group of inventions so linked as to form "a single general inventive concept."

PCT Rule 13.2 indicates that this shall be construed as permitting, in particular, one of the following three possible combinations of the claimed invention:

(1) a product, a process specifically adapted for the manufacture of said product and a use of said product, or

(2) a process, and an apparatus or means specifically designed for carrying out said process, or

(3) a product, a process specially adapted for the manufacture of said product and an apparatus or means designed for carrying out the process.

Additionally, current United States Patent and Trademark Office restriction practice permits the following combinations of the claimed invention:

(4) a product, and a process specifically adapted for the manufacture of said product, and

(5) a product, and a use of the said product, as where said use as claimed cannot be practiced with another materially different product.

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-18, a first method drawn to methods of treating hypertension.

Group II, claims 19-36, a second method drawn to methods of treating hypotension.

Group III, a first specie of TGF-β drawn to TGF-β1.

Group IV, a second specie of TGF-β drawn to TGF-β2.

Group V, a third specie of TGF-β drawn to TGF-β1/β2 hybrids.

Group VI, a fourth specie of TGF-β drawn to TGF-β1 precursor.

Group VII, a fifth specie of TGF-β drawn to TGF-β2 precursor.

Group VIII, a sixth specie of TGF-β drawn to TGF-β1/TGF-β2 precursor.

Group IX, a seventh specie of TGF-β drawn to TGF-β1 complex.

Group X, an eighth specie of TGF-β drawn to TGF-β2 complex.

The inventions listed as Groups I-X do not meet the requirements for Unity of Invention for the following reasons:

The inventions of Groups I and II are directed to methods of treating two pathologic disease states using different reagents and are not so linked as to form a single general inventive concept.

The inventions of Groups III-X are directed to species of TGF-β that differ in physical properties such as chemical composition primary amino acid sequence and molecular weight and are not so linked as to form a single general inventive concept.

During a telephonic requirement for election, on August 8, 1991, applicant's representative, Brian W. Poor, elected the invention of Groups I, III, and the additional Groups V-X for examination.